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Customer No. 35745

Docket No. 100390-9630

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants	:	Kenten et al.
Serial No.	:	09/480,544
Filed	:	January 10, 2000
For	:	Cycling DNA/RNA Amplification Electrochemiluminescent Probe Assay
Group Art Unit	:	1643
Examiner	:	Chakrabartu, Arun K
Confirmation No.	:	4434

CERTIFICATE OF FIRST CLASS MAILING

I hereby certify that this correspondence is being deposited on January 23, 2004, with the United States Postal Service as First Class Mail in an envelope addressed to: Mail Stop Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Date: January 23, 2004

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APPELLANT'S BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This is an appeal from the Official Action dated December 27, 2002, finally rejecting claims 32-43.

The instant brief is submitted in triplicate as required by 37 C.F.R. § 1.192(a).

A check in the amount of $165.00 is also submitted in payment of the fee required by 37 C.F.R. § 1.17(c).

A Petition For Five Months Extension Of Time was concurrently filed to extend the time for filing the Brief until January 25, 2004. Thus, this Brief is timely filed.

REAL PARTY IN INTEREST

The real party in interest in this appeal is the assignee, IGEN International, Inc.

RELATED APPEALS AND INTERFERENCES

Appellants are not aware of any related appeals or interferences that directly affect or are directly affected by or have a bearing on the Board's decision in the pending appeal.

STATUS OF CLAIMS

The application was filed as US Application Serial No. 09/480,544 on January 10, 2000, which is a divisional application of US Application Serial No. 08/474,927 filed on June 7, 1995, now US Patent No. 6,048,687. The subject application was filed with claims 21-31. Claims 21-31 were canceled and claims 32-43 were added in a Amendment mailed December 14, 2001 (hereinafter, "12/14/01 Amendment").

Therefore, claims 32-43 are pending in the instant application.

The claims of this application were finally rejected in an Office Action mailed December 27, 2002.

The status of the claims is as follows:

Allowed claims:	None
Claims objected to:	None
Claims rejected:	32-43.

These claims are set out in Appendix A, attached hereto.

The claims on appeal are 32-43.

STATUS OF AMENDMENTS

No amendments have been filed subsequent to the final rejection.

SUMMARY OF INVENTION

The present invention relates to an improved process for detecting a specific nucleic acid sequence. The process involves the synthesis of single stranded RNA, single stranded DNA, and double stranded DNA using nucleic acid amplification protocol followed by the addition of two oligonucleotide probes--one with a binding species (*i.e.*, the capture probe (e.g., biotin or antigen)) and one with an electrochemiluminescent label for an ECL based detection. (Specification, page 3). Alternatively, one of the probes may be omitted where the first or second primer or at least one of the nucleotides is labeled with the ECL label, or labeled with the binding species. (Specification, page 16).

More specifically, the amplification protocol of the subject invention comprises amplifying a specific nucleic acid sequence by forming a composition comprising (i) a sample, (ii) a first oligonucleotide primer which comprises a promoter sequence, (iii) a second oligonucleotide primer, (iv) a DNA-directed RNA polymerase, (v) an RNA-directed DNA polymerase, (vi) a DNA-directed DNA polymerase, and (vii) a ribonuclease that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA; and incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence.

The amplified product is then combined with (i) at least one detection probe sequence labeled with an electrochemiluminescent species which specifically hybridizes to the amplified nucleic acid sequence, (ii) at least one capture probe sequence labeled with a binding species which specifically hybridizes to the amplified nucleic acid sequence and (iii) a solid phase coated with a binding partner of the binding species; and incubating the reaction mixture for a time sufficient to allow hybridization of probes to amplified nucleic- acid sequence and to allow

binding of binding species to binding partner so as to form a solid phase-bound hybridization complex, which is detected by using electrochemiluminescent species. (Specification, pages 4-6). Optionally, the capture or detection probes may be omitted where the first or second primer or at least one of the nucleotides is labeled with the ECL label, or labeled with the binding species. (Specification, page 16).

The present invention requires no pretreatment of the sample such as binding to solid phases or membranes, denaturing of the sample, purification of the sample by extraction of oil, protein or by gel electrophoresis. Using the invention, the probes can be added directly to the amplification mixture, hybridized and analyzed. This is surprising in light of the belief that the probe sequences would be modified or sample would be destroyed by the enzymes and conditions in the amplification mixture. For example, one skilled in the art would have believed that the presence of RNAase H, which degrades hybrids between DNA and RNA (the basis of the probe hybridization), would degrade the specific hybrids in any attempt to probe the impure amplification mix and that the presence of reverse transcriptase would use the probes in the hybridization mixture as primers and remove them from the specific hybridization complex formation reaction.

In addition to these potential problems, the buffer contains many compounds which would have been expected to cause problems both for hybridization and also for the generation of ECL because of the specific chemistries involved, for example, high levels of salts $MgCl_2$, KCl, nucleotides, dithiothreitol, spermidine, dimethyl sulphoxide, glycerol and proteins. This list contains many substances which interfere with other nucleic acid probe methods and removal of them would have been believed to be necessary to allow these methods to work. Thus, the

present invention was surprising in that it is capable of carrying out a nucleic acid probe assay with such a simple protocol. (Specification, page 3).

ISSUES

Whether the subject matter of claims 32-43 are obvious over the disclosure of U.S. Patent No. 5,130,238 to Malek (hereinafter, "Malek") in view of U.S. Patent No. 6,174,709 to Kenten et al. (hereinafter, "Kenten") under 35 U.S.C. § 103 (a).

GROUPING OF CLAIMS

Appellants urge that claims 32-43 do not stand or fall together and request grouping the instant claims into two separate groups: (1) claims 32-37 and (2) claims 38-43.

Claim 32 is directed to a process for the detection of a specific nucleic acid sequence, which requires both the use of detection and capture probe sequences. Claims 33-37 are dependent on claim 32.

Claim 38 differs from claim 32 in that it allows for the omission of the detection or capture probe. Claims 39-43 are dependent on claim 38.

Accordingly, claims 32-37 and claims 38-43 are technically different and patentably distinct. Appellants respectfully request that the alleged obviousness of the two claim groups be analyzed separately.

ARGUMENT

The teachings of Malek, alone or in combination with the teachings of Kenten do not teach or suggest the presently claimed subject matter. Claims 32-43 are patentable over cited references.

The Examiner correctly states that "one cannot show nonobviousness by attacking references individually where the rejection is based on combination of references" (Advisory

Action, page 1). However, Appellants herein demonstrate the deficiency in the Malek reference, which is not compensated by the disclosure of Kenten. Therefore the cited references alone or in combination do not teach or suggest the present invention.

1. Malek and Kenten do not suggest or teach all of the claim limitations of claims 32-37 (Group 1)

Malek does not teach or suggest the presently claimed subject matter of claims 32-37. Malek merely teaches a few variations of the nucleic acid sequence based amplification (NASBA) protocol. Nothing in the disclosure of Malek teaches or suggests that an ECL probe can be used in NASBA amplification. Neither does Malek teach or suggest using a capture probe labeled with a binding species and a bead coated with binding species complementary to the capture probe. Moreover, the detection method of Malek is autoradiography.

All of the deficiencies of Malek are admitted by the Examiner (Office Action, page 6). The Examiner, however, asserts that it would have been obvious to modify the teachings of Malek in view of the teachings of Kenten to result in the presently claimed invention.

Appellants respectfully submit that Kenten does not compensate for the deficiencies of Malek. More specifically, the detection method of Kenten is not the detection method of the subject application.

Appellants urge that the claimed embodiment of the invention (the detection of unlabeled amplification products through the use of two probes, one having an ECL label and the other having a capture moiety claimed in independent claim 32 (c-e) is not disclosed in the Malek reference alone or in combination with the Kenten reference. Kenten merely discloses the combination of a labeled primer and a single labeled probe, while the method of Malek employs autoradiography. Kenten does not teach or suggest the detection of unlabeled amplification

products through the use of two probes: a first probe having an ECL label and the other probe having a capture moiety.

The Examiner's attempt to rebut Appellants arguments that the detection method of Kenten is not the detection method of subject claims on page 1 of the Advisory Action appears to be factually incorrect. Example I of Kenten merely teaches the chemical synthesis of an ECL label. The Appellants were unable to find where "Kenten clearly teaches the addition of: (I) at least one probe sequence complementary to the RNA first template labeled with an electrochemiluminescent species comprising ruthenium-tris-bipyridine (Example I)" (Advisory Action, page 1) in Example I of Kenten. Furthermore, nowhere in Kenten does it teach the addition to an amplified product of two probes: one labeled with a capture species and the other with an ECL label.

Thus, it would not have been obvious to one of ordinary skill in the art to modify Malek in view of Kenten since (1) the combination of the detection method of Kenten with the amplification method of Malek doesn't disclose every element of the subject claims and does not add up to the present invention and/or (2) there is no motivation to combine to combine the teachings of Malek with Kenten, as detailed below.

Therefore, Malek, alone, or in combination with Kenten, does not teach or suggest all of the limitations of the currently pending claims and therefore do not teach the subject invention as a whole. The MPEP clearly states:

> To establish *prima facie* obviousness of a claimed invention, **all the claim limitations must be taught or suggested by the prior art**. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending

> therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

[See MPEP 2143.03, emphasis added].

The MPEP also clearly states that the prior art must teach or suggest invention as a whole:

> In determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983).
>
> The court held "due to the admitted unobviousness of the first two steps of the claimed combination of steps, the subject matter as a whole would not have been obvious to one of ordinary skill in the art at the time the invention was made." 535 F.2d at 69, 190 USPQ at 17 (emphasis in original).

[MPEP 2141.02].

To summarize, each of the cited references fails to disclose the described limitation of the claims on Appeal and the cited references alone or in combination fail to teach or suggest the subject invention as a whole. Therefore the rejection is improper and should be reversed.

The Examiner's allegation that: "In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., (a) the invention does not require sample pretreatment and (b) the detection of unlabeled amplification products through the use of two probes, one having ECL label and the other having a capture moiety) are not recited in the claims" (Office Action, page 9) is factually incorrect.

For example, contrary to the Examiner's argument in "(a)", the mixtures of the subject invention are formed by direct addition of the probes to the amplification reaction mixture without the removal of the amplification enzymes prior to the addition of the probes. *See* Claim

32 (Group 1) and Claim 38 (Group 2). Applicants note that while Claim 38 defines a patentably distinct group because an additional limitation elsewhere in the body of that claim allows for the omission of the detection or capture probe, the same argument is nevertheless applicable to Claim 38 since it contains the same claim limitations at issue.

Second, contrary to the Examiner's argument "(b)", the detection of unlabeled amplification products through the use of two probes, one having ECL label and the other having a capture moiety is specifically recited in claim 32 step (c):

> Claim 32. [...]
>
> (c) forming a second mixture by adding to a sample of said amplified nucleic acid sequence mixture the following reagents
>
> (i) **at least one detection probe sequence** which specifically hybridizes to said amplified nucleic acid sequence, said detection probe sequence being **labeled with an electrochemiluminescent species,**
>
> (ii) **at least one capture probe sequence** which specifically hybridizes to said amplified nucleic acid sequence, said capture probe sequence being **labeled with a binding species,** and[...]

[Claim 32, emphasis added]; *See also* Claim 38(c) (*i.e.*, once again, as explained in the preceding paragraph, this argument is applicable to Claim 38 since the claim limitations which make the Examiner's argument factually incorrect are present).

Therefore, the rejection of claims 32-37 should be reversed for the reasons set forth above.

2. There is no motivation to combine Malek and Kenten therefore both claims 32-37 and claims 38-43 (Groups 1 and 2) are not obvious.

As admitted by the Examiner (Office Action, page 6), Malek does not teach or suggest the desirability of using ECL labels and ECL detection.

Furthermore, Kenten does not teach or suggest the use of ECL probes in the NASBA amplification of Malek. The suggestion to combine the elements must come from the reference cited and not from the applicant's disclosure. *See In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). To establish prima facie obviousness based on a combination of references, the Examiner is required to demonstrate that the prior art provide "a reason, suggestion, or motivation to lead an inventor to combine those references." *Pro-Mold and Tool Co. v. Great Lakes Plastics Inc.*, 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996).

> [E]vidence of a suggestion, teaching, or motivation to combine may flow from the prior art references themselves, the knowledge of one of ordinary skill in the art, or in some cases, from nature of the problem solved. ... The range of sources available, however, **does not diminish the requirements for actual evidence. That is, the showing must be clear and particular**.

In re Dembiczak, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) (Citation omitted, emphasis added).

There is no motivation or suggestion in the cited references to make the combination suggested by the Examiner. *See* MPEP 2143.01.

> Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. **"The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art."** *In re Kotzab,* 217 F.3d 1365, 1370, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). See also *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

[MPEP 2143.01, emphasis added].

Although Kenten discloses the use of ECL probes in PCR-based single primer amplification, the differences between NASBA and PCR reactions are substantial and the skilled artisan would not reasonably expect that the successful incorporation of ECL technology with PCR would be a good predictor of success with NASBA.

More specifically, the fact that the amplification mixture does not require pretreatment before the probes are added is surprising and unexpected because, at a minimum, the probe sequence could be modified by the very enzymes and components required in the amplification medium. These components are not present in the simpler amplification mixture of Kenten. According to the present invention, three separate enzymes (*i.e.*, reverse transcriptase, RNase H, and an RNA polymerase) are present simultaneously in the assay, which creates a greater likelihood of unwanted side reactions taking place without pretreatment of the sample to remove contaminants. PCR assays use a much simpler mix of enzymes that would be less likely to interfere with the reaction and require, at a minimum, denaturation of the double stranded product.

A person of ordinary skill in the art would expect hybridization efficiencies to RNA in an isothermal NASBA system to be quite low, especially when two hybridizations are required, because of the lack of denaturation step and the complex structure and folding of RNA molecules known in the art. The intramolecular hybridization within the RNA strand makes hybridization to external probes more difficult. In addition, the lower stability of RNA relative to DNA might have been expected to negatively interfere with the detection of the amplification product. Moreover, Malek does not teach or suggest providing conditions of temperature and buffer to allow the hybridization of the probe and an RNA template and the binding of the

binding species on the capture probe with the complementary binding species on the bead to form a labeled bead bound complex and detecting the bead bound complex using ECL detection.

The Examiner discusses "reasonable expectation of success" and concludes: "There is no evidence of record submitted by applicant demonstrating the absence of a reasonable expectation of success. There is evidence in the Kenten reference [...]" (Office Action, page 9).

The Examiner has failed to specifically point out where in the Kenten reference could a person of ordinary skill in the art find suggestion or motivation to use the detection method of Kenten with the NASBA amplification. Instead, the Examiner relies on a citation from Kenten taken out of context. The citation does not teach or suggest the desirability of making a combination suggested by the Examiner. More specifically, the Examiner cites Kenten in support of the rejection: "The unexpected exponential amplification of the invention greatly simplifies the process of amplifying multiple nucleic acid sequences of interest present in a sample (Column 5, lines 1-4)" (Office Action, page 8). This citation is grossly misinterpreted.

Kenten teaches a PCR amplification using a single unpaired primer, where the amplification is expected to be linear and not exponential. The amplification which is expected to be linear and slow accelerates at some point during the process and becomes exponential. This fact is advantageous for the single primer amplification, as it reduces the total amplification time and reduces the possibility of amplification errors. In reviewing prior art, Kenten states:

> [...] if a single unpaired primer is used in place of two (paired) primers, the result is a linear growth in extension product copy number instead of an exponential growth of both strands (3). It is generally believed that the reason for the linear growth in copy number with cycle using a single unpaired primer is that only the template strand is replicated during each cycle. The primer extension itself is not copied.

[Kenten, col. 1, lines 54-61].

Kenten specifically teaches that an exponential amplification using a single unpaired primer is the advantageous discovery of his invention:

> [...] heretofore unavailable method for achieving exponential amplification of a specific nucleic acid sequence of interest requiring only a single primer but retaining specificity of action would be an important and unexpected contribution to the art.

[Kenten, col. 4, lines 8-12].

The unexpected exponential character of the single primer PCR amplification in Kenten can not be viewed as a suggestion or motivation to combine the ECL labels of Kenten with the NASBA amplification of Malek. Kenten does not teach or suggest that a single primer PCR amplification is in any way related to or can be a substitute for NASBA, or that such a substitution may be desirable. Although Kenten discloses the use of ECL primers in PCR-based single primer amplification, the differences between NASBA and PCR reactions are substantial and the skilled artisan would not reasonably expect that the successful incorporation of ECL technology with one would be a good predictor of success with the other.

Thus, Appellants urge that the Examiner finds the entire support for a suggestion or motivation to combine these references in a citation taken out of context. Therefore, Appellants urge that there is no suggestion or motivation to combine the teachings of Malek with the teachings of Kenten. The Examiner fails to provide the required factual support to establish a prima facie case of obviousness.

In addition, the Examiner's assertion that PCR can yield RNA products using RNA polymerase is immaterial to the patentability of the subject invention (Advisory Action, page 2). The Kenten reference explicitly teaches PCR reactions for DNA to DNA and RNA to DNA amplification. The desirability (or feasibility) of using PCR amplification, which produces RNA products, combined with ECL detection is not taught or suggested in the Kenten reference.

There is no motivation or suggestion in the cited references to make the combination suggested by the Examiner. *See* MPEP 2143.01. More specifically, the Examiner fails to provide a factual support for the suggestion or motivation for using a detection method of Kenten with the NASBA amplification of Malek. Absent the explicit suggestion to combine or combination of the cited references the rejection is improper and should be reversed.

3. Summary

To summarize, Appellants maintain the non-obviousness of claims 32-43 of the current U.S. Application No. 09/480,544 over the teachings of Malek in view of Kenten.

As stated in the MPEP:

> When applying 35 U.S.C. 103, the following tenets of patent law must be adhered to:
> (A) The claimed invention must be considered as a whole;
> (B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination;
> (C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and
> (D) Reasonable expectation of success is the standard with which obviousness is determined.
> *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986).

[MPEP 2141].

With respect to (A), Appellants urge that Malek, alone or in combination with Kenten, does not teach or suggest the claimed subject matter as a whole. None of the references cited by the Examiner teach the use NASBA amplification protocol with ECL detection technology. Furthermore, none of the cited references teach or suggest the detection which uses a combination of detection and capture probes.

With respect to (B), the references do not teach or suggest the desirability of making the combination as discussed above. Briefly, Malek does not teach or suggest the desirability of using ECL detection with NASBA amplification and Kenten does not teach or suggest the desirability of using NASBA amplification with ECL detection. The differences between NASBA and PCR reactions are substantial and the skilled artisan would not reasonably expect that the successful incorporation of ECL technology with PCR would be a good predictor of success with NASBA. Thus, one of ordinary skill in the art would not have the motivation to combine teachings of Malek and Kenten for the reasons set forth above.

With respect to (C), there is no motivation or suggestion in any of the references to make the combination suggested in the Final Office Action.

With respect to (D), there is no reasonable expectation of success in making a combination suggested by the Examiner due to the differences between NASBA and PCR reactions as discussed above.

Favorable reconsideration and withdrawal of the Section 103(a) rejection are earnestly solicited.

CONCLUSION

In view of the foregoing, Appellants respectfully submit that the instant claims are non-obvious over Malek in view of Kenten. Thus, the claims are in condition for allowance and such action is earnestly solicited.

Dated: January 23, 2004

Respectfully submitted,

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APPENDIX A

32. A process for the detection of a specific nucleic acid sequence comprising:

(a) forming a composition comprising

(i) a sample,

(ii) a first oligonucleotide primer which comprises a promoter sequence,

(iii) a second oligonucleotide primer,

(iv) a DNA-directed RNA polymerase,

(v) an RNA-directed DNA polymerase,

(vi) a DNA-directed DNA polymerase, and

(vii) a ribonuclease that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA;

(b) incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence to form an amplified nucleic acid sequence mixture comprising an amplified nucleic acid sequence;

(c) forming a second mixture by adding to a sample of said amplified nucleic acid sequence mixture the following reagents

(i) at least one detection probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said detection probe sequence being labeled with an electrochemiluminescent species,

(ii) at least one capture probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said capture probe sequence being labeled with a binding species, and

(iii) a solid phase coated with a binding partner of said binding species;

(d) incubating said second mixture for a time sufficient to allow hybridization of said probes to said amplified nucleic- acid sequence and to allow binding of said binding species to said binding partner so as to form a solid phase-bound hybridization complex; and

(e) detecting said solid phase-bound complex by using said electrochemiluminescent species.

33. The process of claim 32, wherein said solid phase is a magnetic bead.

34. The process of claim 32, wherein the binding species/binding partner pair are selected from the group consisting of biotin/avidin, biotin/streptavidin, and digoxigenin/anti-digoxigenenin.

35. The process of claim 32, wherein the binding species is biotin and the solid phase is a streptavidin-coated magnetic bead.

36. The process of claim 32, wherein said amplified nucleic acid sequence is the anti-sense copy of the specific nucleic acid sequence and wherein said amplification of said specific nucleic acid sequence is carried out under conditions which permit

(i) said second oligonucleotide primer to hybridize to an RNA template which comprises the specific nucleic acid sequence or an anti-sense copy of the specific nucleic acid sequence,

(ii) said RNA-directed DNA polymerase to utilize said RNA template to synthesize a DNA template by extension of said second oligonucleotide primer and thereby form an RNA-DNA hybrid intermediate,

(iii) said ribonuclease to hydrolyze RNA contained in said RNA-DNA hybrid intermediate,

(iv) said first oligonucleotide primer to hybridize to said DNA template,

(v) said DNA-directed DNA polymerase to utilize said DNA template to synthesize a double-stranded DNA product by extension of said first olignucleotide primer, said double stranded DNA product comprising said promoter, and

(vi) said DNA-directed RNA polymerase to recognize said promoter and transcribe said double- stranded DNA product so as to form more RNA first template.

37. The process of claim 32, wherein said electrochemihuninescent species comprises ruthenium-tris-bipyridine.

38. A process for the detection of a specific nucleic acid sequence, comprising:

(a) forming a composition comprising:

(i) the sample,

(ii) a first oligonucleotide primer which comprises a promoter sequence,

(iii) a second oligonucleotide primer,

(iv) a DNA-directed RNA polymerase,

(v) an RNA-directed DNA polymerase,

(vi) a DNA-directed DNA polymerase,

(vii) a ribonuclease that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, and

(viii) one or more nucleotides;

(b) incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence to form an amplified nucleic acid sequence mixture comprising an amplified nucleic acid sequence;

(c) forming a second mixture by adding to a sample of said amplified nucleic acid sequence mixture the following reagents

(i) at least one detection probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said detection probe sequence being labeled with an electrochemiluminescent species,

(ii) at least one capture probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said capture probe sequence being labeled with a binding species, and

(iii) a solid phase coated with a binding partner of said binding species;

(d) incubating said second mixture for a time sufficient to allow hybridization between said probes and to allow binding of said binding species to said binding partner so as to form a solid phase-bound hybridization complex; and

(e) detecting said solid phase-bound complex by using said electrochemiluminescent species;

wherein, optionally, said first primer, second primer or at least one of said nucleotides is labeled with the electrochemiluminescent species and said detection probe is omitted, and, optionally, said first primer, second primer or at least a portion of said nucleotides is labeled with the binding species and said capture-probe is omitted.

39. The process of claim 38, wherein the solid phase is a magnetic bead.

40. The process of claim 38, wherein the binding species/binding partner pair are selected from the group consisting of biotin/avidin, biotin/streptavidin, and digoxigenin/anti-digoxigenenin.

41. The process of claim 38, wherein the binding species is biotin and the solid phase is a streptavidin-coated magnetic bead.

42. The process of claim 38, wherein said amplified nucleic acid sequence is the anti-sense copy of the specific nucleic acid sequence and wherein said amplification of said specific nucleic acid sequence is carried out under conditions which permit

(i) said second oligonucleotide primer to hybridize to an RNA template which comprises the specific nucleic acid sequence or an anti-sense copy of the specific nucleic acid sequence,

(ii) said RNA-directed DNA polymerase to utilize said RNA template to synthesize a DNA template by extension of said second oligonucleotide primer and thereby form an RNA-DNA hybrid intermediate,

(iii) said ribonuclease to hydrolyze RNA contained in said RNA-DNA hybrid intermediate,

(iv) said first oligonucleotide primer to hybridize to said DNA template,

(v) said DNA-directed DNA polymerase to utilize said DNA template to synthesize a double-stranded DNA product by extension of said first oligonucleotide primer, said double stranded DNA product comprising said promoter, and

(vi) said DNA-directed RNA polymerase to recognize said promoter and transcribe said double stranded DNA product so as to form more RNA first template.

43. The process of claim 38, wherein said electrochemiluminescent species comprises ruthenium-tris-bipyridine.

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CERTIFICATE OF FIRST CLASS MAILING

I hereby certify that this correspondence is being deposited on January 23, 2004, with the United States Postal Service as First Class Mail in an envelope addressed to: Mail Stop Appeal Brief - Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Date: January 23, 2004

Eleanor N. Johnson, Reg. No. 53,076

APPELLANT'S BRIEF

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The claims of this application were finally rejected in an Office Action mailed December 27, 2002.

The status of the claims is as follows:

Allowed claims:	None
Claims objected to:	None
Claims rejected:	32-43.

These claims are set out in Appendix A, attached hereto.

The claims on appeal are 32-43.

STATUS OF AMENDMENTS

No amendments have been filed subsequent to the final rejection.

SUMMARY OF INVENTION

The present invention relates to an improved process for detecting a specific nucleic acid sequence. The process involves the synthesis of single stranded RNA, single stranded DNA, and double stranded DNA using nucleic acid amplification protocol followed by the addition of two oligonucleotide probes--one with a binding species (*i.e.*, the capture probe (e.g., biotin or antigen)) and one with an electrochemiluminescent label for an ECL based detection. (Specification, page 3). Alternatively, one of the probes may be omitted where the first or second primer or at least one of the nucleotides is labeled with the ECL label, or labeled with the binding species. (Specification, page 16).

More specifically, the amplification protocol of the subject invention comprises amplifying a specific nucleic acid sequence by forming a composition comprising (i) a sample, (ii) a first oligonucleotide primer which comprises a promoter sequence, (iii) a second oligonucleotide primer, (iv) a DNA-directed RNA polymerase, (v) an RNA-directed DNA polymerase, (vi) a DNA-directed DNA polymerase, and (vii) a ribonuclease that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA; and incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence.

The amplified product is then combined with (i) at least one detection probe sequence labeled with an electrochemiluminescent species which specifically hybridizes to the amplified nucleic acid sequence, (ii) at least one capture probe sequence labeled with a binding species which specifically hybridizes to the amplified nucleic acid sequence and (iii) a solid phase coated with a binding partner of the binding species; and incubating the reaction mixture for a time sufficient to allow hybridization of probes to amplified nucleic- acid sequence and to allow

binding of binding species to binding partner so as to form a solid phase-bound hybridization complex, which is detected by using electrochemiluminescent species. (Specification, pages 4-6). Optionally, the capture or detection probes may be omitted where the first or second primer or at least one of the nucleotides is labeled with the ECL label, or labeled with the binding species. (Specification, page 16).

The present invention requires no pretreatment of the sample such as binding to solid phases or membranes, denaturing of the sample, purification of the sample by extraction of oil, protein or by gel electrophoresis. Using the invention, the probes can be added directly to the amplification mixture, hybridized and analyzed. This is surprising in light of the belief that the probe sequences would be modified or sample would be destroyed by the enzymes and conditions in the amplification mixture. For example, one skilled in the art would have believed that the presence of RNAase H, which degrades hybrids between DNA and RNA (the basis of the probe hybridization), would degrade the specific hybrids in any attempt to probe the impure amplification mix and that the presence of reverse transcriptase would use the probes in the hybridization mixture as primers and remove them from the specific hybridization complex formation reaction.

In addition to these potential problems, the buffer contains many compounds which would have been expected to cause problems both for hybridization and also for the generation of ECL because of the specific chemistries involved, for example, high levels of salts $MgCl_2$, KCl, nucleotides, dithiothreitol, spermidine, dimethyl sulphoxide, glycerol and proteins. This list contains many substances which interfere with other nucleic acid probe methods and removal of them would have been believed to be necessary to allow these methods to work. Thus, the

present invention was surprising in that it is capable of carrying out a nucleic acid probe assay with such a simple protocol. (Specification, page 3).

ISSUES

Whether the subject matter of claims 32-43 are obvious over the disclosure of U.S. Patent No. 5,130,238 to Malek (hereinafter, "Malek") in view of U.S. Patent No. 6,174,709 to Kenten et al. (hereinafter, "Kenten") under 35 U.S.C. § 103 (a).

GROUPING OF CLAIMS

Appellants urge that claims 32-43 do not stand or fall together and request grouping the instant claims into two separate groups: (1) claims 32-37 and (2) claims 38-43.

Claim 32 is directed to a process for the detection of a specific nucleic acid sequence, which requires both the use of detection and capture probe sequences. Claims 33-37 are dependent on claim 32.

Claim 38 differs from claim 32 in that it allows for the omission of the detection or capture probe. Claims 39-43 are dependent on claim 38.

Accordingly, claims 32-37 and claims 38-43 are technically different and patentably distinct. Appellants respectfully request that the alleged obviousness of the two claim groups be analyzed separately.

ARGUMENT

The teachings of Malek, alone or in combination with the teachings of Kenten do not teach or suggest the presently claimed subject matter. Claims 32-43 are patentable over cited references.

The Examiner correctly states that "one cannot show nonobviousness by attacking references individually where the rejection is based on combination of references" (Advisory

Action, page 1). However, Appellants herein demonstrate the deficiency in the Malek reference, which is not compensated by the disclosure of Kenten. Therefore the cited references alone or in combination do not teach or suggest the present invention.

1. Malek and Kenten do not suggest or teach all of the claim limitations of claims 32-37 (Group 1)

Malek does not teach or suggest the presently claimed subject matter of claims 32-37. Malek merely teaches a few variations of the nucleic acid sequence based amplification (NASBA) protocol. Nothing in the disclosure of Malek teaches or suggests that an ECL probe can be used in NASBA amplification. Neither does Malek teach or suggest using a capture probe labeled with a binding species and a bead coated with binding species complementary to the capture probe. Moreover, the detection method of Malek is autoradiography.

All of the deficiencies of Malek are admitted by the Examiner (Office Action, page 6). The Examiner, however, asserts that it would have been obvious to modify the teachings of Malek in view of the teachings of Kenten to result in the presently claimed invention.

Appellants respectfully submit that Kenten does not compensate for the deficiencies of Malek. More specifically, the detection method of Kenten is not the detection method of the subject application.

Appellants urge that the claimed embodiment of the invention (the detection of unlabeled amplification products through the use of two probes, one having an ECL label and the other having a capture moiety claimed in independent claim 32 (c-e) is not disclosed in the Malek reference alone or in combination with the Kenten reference. Kenten merely discloses the combination of a labeled primer and a single labeled probe, while the method of Malek employs autoradiography. Kenten does not teach or suggest the detection of unlabeled amplification

products through the use of two probes: a first probe having an ECL label and the other probe having a capture moiety.

The Examiner's attempt to rebut Appellants arguments that the detection method of Kenten is not the detection method of subject claims on page 1 of the Advisory Action appears to be factually incorrect. Example I of Kenten merely teaches the chemical synthesis of an ECL label. The Appellants were unable to find where "Kenten clearly teaches the addition of: (I) at least one probe sequence complementary to the RNA first template labeled with an electrochemiluminescent species comprising ruthenium-tris-bipyridine (Example I)" (Advisory Action, page 1) in Example I of Kenten. Furthermore, nowhere in Kenten does it teach the addition to an amplified product of two probes: one labeled with a capture species and the other with an ECL label.

Thus, it would not have been obvious to one of ordinary skill in the art to modify Malek in view of Kenten since (1) the combination of the detection method of Kenten with the amplification method of Malek doesn't disclose every element of the subject claims and does not add up to the present invention and/or (2) there is no motivation to combine to combine the teachings of Malek with Kenten, as detailed below.

Therefore, Malek, alone, or in combination with Kenten, does not teach or suggest all of the limitations of the currently pending claims and therefore do not teach the subject invention as a whole. The MPEP clearly states:

> To establish *prima facie* obviousness of a claimed invention, **all the claim limitations must be taught or suggested by the prior art**. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). If an independent claim is nonobvious under 35 U.S.C. 103, then any claim depending

> therefrom is nonobvious. *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

[See MPEP 2143.03, emphasis added].

The MPEP also clearly states that the prior art must teach or suggest invention as a whole:

> In determining the differences between the prior art and the claims, the question under 35 U.S.C. 103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir. 1983); *Schenck v. Nortron Corp.*, 713 F.2d 782, 218 USPQ 698 (Fed. Cir. 1983).
>
> The court held "due to the admitted unobviousness of the first two steps of the claimed combination of steps, the subject matter as a whole would not have been obvious to one of ordinary skill in the art at the time the invention was made." 535 F.2d at 69, 190 USPQ at 17 (emphasis in original).

[MPEP 2141.02].

To summarize, each of the cited references fails to disclose the described limitation of the claims on Appeal and the cited references alone or in combination fail to teach or suggest the subject invention as a whole. Therefore the rejection is improper and should be reversed.

The Examiner's allegation that: "In response to applicant's argument that the references fail to show certain features of applicant's invention, it is noted that the features upon which applicant relies (i.e., (a) the invention does not require sample pretreatment and (b) the detection of unlabeled amplification products through the use of two probes, one having ECL label and the other having a capture moiety) are not recited in the claims" (Office Action, page 9) is factually incorrect.

For example, contrary to the Examiner's argument in "(a)", the mixtures of the subject invention are formed by direct addition of the probes to the amplification reaction mixture without the removal of the amplification enzymes prior to the addition of the probes. *See* Claim

32 (Group 1) and Claim 38 (Group 2). Applicants note that while Claim 38 defines a patentably distinct group because an additional limitation elsewhere in the body of that claim allows for the omission of the detection or capture probe, the same argument is nevertheless applicable to Claim 38 since it contains the same claim limitations at issue.

Second, contrary to the Examiner's argument "(b)", the detection of unlabeled amplification products through the use of two probes, one having ECL label and the other having a capture moiety is specifically recited in claim 32 step (c):

> Claim 32. [...]
>
> (c) forming a second mixture by adding to a sample of said amplified nucleic acid sequence mixture the following reagents
>
> (i) **at least one detection probe sequence** which specifically hybridizes to said amplified nucleic acid sequence, said detection probe sequence being **labeled with an electrochemiluminescent species,**
>
> (ii) **at least one capture probe sequence** which specifically hybridizes to said amplified nucleic acid sequence, said capture probe sequence being **labeled with a binding species,** and[...]

[Claim 32, emphasis added]; *See also* Claim 38(c) (*i.e.*, once again, as explained in the preceding paragraph, this argument is applicable to Claim 38 since the claim limitations which make the Examiner's argument factually incorrect are present).

Therefore, the rejection of claims 32-37 should be reversed for the reasons set forth above.

2. There is no motivation to combine Malek and Kenten therefore both claims 32-37 and claims 38-43 (Groups 1 and 2) are not obvious.

As admitted by the Examiner (Office Action, page 6), Malek does not teach or suggest the desirability of using ECL labels and ECL detection.

Furthermore, Kenten does not teach or suggest the use of ECL probes in the NASBA amplification of Malek. The suggestion to combine the elements must come from the reference cited and not from the applicant's disclosure. *See In re Dow Chemical Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). To establish prima facie obviousness based on a combination of references, the Examiner is required to demonstrate that the prior art provide "a reason, suggestion, or motivation to lead an inventor to combine those references." *Pro-Mold and Tool Co. v. Great Lakes Plastics Inc.*, 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1629 (Fed. Cir. 1996).

> [E]vidence of a suggestion, teaching, or motivation to combine may flow from the prior art references themselves, the knowledge of one of ordinary skill in the art, or in some cases, from nature of the problem solved. ... The range of sources available, however, **does not diminish the requirements for actual evidence. That is, the showing must be clear and particular**.

In re Dembiczak, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) (Citation omitted, emphasis added).

There is no motivation or suggestion in the cited references to make the combination suggested by the Examiner. *See* MPEP 2143.01.

> Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. **"The test for an implicit showing is what the combined teachings, knowledge of one of ordinary skill in the art, and the nature of the problem to be solved as a whole would have suggested to those of ordinary skill in the art."** *In re Kotzab,* 217 F.3d 1365, 1370, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000). See also *In re Fine*, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988); *In re Jones*, 958 F.2d 347, 21 USPQ2d 1941 (Fed. Cir. 1992).

[MPEP 2143.01, emphasis added].

Although Kenten discloses the use of ECL probes in PCR-based single primer amplification, the differences between NASBA and PCR reactions are substantial and the skilled artisan would not reasonably expect that the successful incorporation of ECL technology with PCR would be a good predictor of success with NASBA.

More specifically, the fact that the amplification mixture does not require pretreatment before the probes are added is surprising and unexpected because, at a minimum, the probe sequence could be modified by the very enzymes and components required in the amplification medium. These components are not present in the simpler amplification mixture of Kenten. According to the present invention, three separate enzymes (*i.e.*, reverse transcriptase, RNase H, and an RNA polymerase) are present simultaneously in the assay, which creates a greater likelihood of unwanted side reactions taking place without pretreatment of the sample to remove contaminants. PCR assays use a much simpler mix of enzymes that would be less likely to interfere with the reaction and require, at a minimum, denaturation of the double stranded product.

A person of ordinary skill in the art would expect hybridization efficiencies to RNA in an isothermal NASBA system to be quite low, especially when two hybridizations are required, because of the lack of denaturation step and the complex structure and folding of RNA molecules known in the art. The intramolecular hybridization within the RNA strand makes hybridization to external probes more difficult. In addition, the lower stability of RNA relative to DNA might have been expected to negatively interfere with the detection of the amplification product. Moreover, Malek does not teach or suggest providing conditions of temperature and buffer to allow the hybridization of the probe and an RNA template and the binding of the

binding species on the capture probe with the complementary binding species on the bead to form a labeled bead bound complex and detecting the bead bound complex using ECL detection.

The Examiner discusses "reasonable expectation of success" and concludes: "There is no evidence of record submitted by applicant demonstrating the absence of a reasonable expectation of success. There is evidence in the Kenten reference [...]" (Office Action, page 9).

The Examiner has failed to specifically point out where in the Kenten reference could a person of ordinary skill in the art find suggestion or motivation to use the detection method of Kenten with the NASBA amplification. Instead, the Examiner relies on a citation from Kenten taken out of context. The citation does not teach or suggest the desirability of making a combination suggested by the Examiner. More specifically, the Examiner cites Kenten in support of the rejection: "The unexpected exponential amplification of the invention greatly simplifies the process of amplifying multiple nucleic acid sequences of interest present in a sample (Column 5, lines 1-4)" (Office Action, page 8). This citation is grossly misinterpreted.

Kenten teaches a PCR amplification using a single unpaired primer, where the amplification is expected to be linear and not exponential. The amplification which is expected to be linear and slow accelerates at some point during the process and becomes exponential. This fact is advantageous for the single primer amplification, as it reduces the total amplification time and reduces the possibility of amplification errors. In reviewing prior art, Kenten states:

> [...] if a single unpaired primer is used in place of two (paired) primers, the result is a linear growth in extension product copy number instead of an exponential growth of both strands (3). It is generally believed that the reason for the linear growth in copy number with cycle using a single unpaired primer is that only the template strand is replicated during each cycle. The primer extension itself is not copied.

[Kenten, col. 1, lines 54-61].

Kenten specifically teaches that an exponential amplification using a single unpaired primer is the advantageous discovery of his invention:

> [...] heretofore unavailable method for achieving exponential amplification of a specific nucleic acid sequence of interest requiring only a single primer but retaining specificity of action would be an important and unexpected contribution to the art.

[Kenten, col. 4, lines 8-12].

The unexpected exponential character of the single primer PCR amplification in Kenten can not be viewed as a suggestion or motivation to combine the ECL labels of Kenten with the NASBA amplification of Malek. Kenten does not teach or suggest that a single primer PCR amplification is in any way related to or can be a substitute for NASBA, or that such a substitution may be desirable. Although Kenten discloses the use of ECL primers in PCR-based single primer amplification, the differences between NASBA and PCR reactions are substantial and the skilled artisan would not reasonably expect that the successful incorporation of ECL technology with one would be a good predictor of success with the other.

Thus, Appellants urge that the Examiner finds the entire support for a suggestion or motivation to combine these references in a citation taken out of context. Therefore, Appellants urge that there is no suggestion or motivation to combine the teachings of Malek with the teachings of Kenten. The Examiner fails to provide the required factual support to establish a prima facie case of obviousness.

In addition, the Examiner's assertion that PCR can yield RNA products using RNA polymerase is immaterial to the patentability of the subject invention (Advisory Action, page 2). The Kenten reference explicitly teaches PCR reactions for DNA to DNA and RNA to DNA amplification. The desirability (or feasibility) of using PCR amplification, which produces RNA products, combined with ECL detection is not taught or suggested in the Kenten reference.

There is no motivation or suggestion in the cited references to make the combination suggested by the Examiner. *See* MPEP 2143.01. More specifically, the Examiner fails to provide a factual support for the suggestion or motivation for using a detection method of Kenten with the NASBA amplification of Malek. Absent the explicit suggestion to combine or combination of the cited references the rejection is improper and should be reversed.

3. Summary

To summarize, Appellants maintain the non-obviousness of claims 32-43 of the current U.S. Application No. 09/480,544 over the teachings of Malek in view of Kenten.

As stated in the MPEP:

> When applying 35 U.S.C. 103, the following tenets of patent law must be adhered to:
> (A) The claimed invention must be considered as a whole;
> (B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination;
> (C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and
> (D) Reasonable expectation of success is the standard with which obviousness is determined.
> *Hodosh v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143 n.5, 229 USPQ 182, 187 n.5 (Fed. Cir. 1986).

[MPEP 2141].

With respect to (A), Appellants urge that Malek, alone or in combination with Kenten, does not teach or suggest the claimed subject matter as a whole. None of the references cited by the Examiner teach the use NASBA amplification protocol with ECL detection technology. Furthermore, none of the cited references teach or suggest the detection which uses a combination of detection and capture probes.

With respect to (B), the references do not teach or suggest the desirability of making the combination as discussed above. Briefly, Malek does not teach or suggest the desirability of using ECL detection with NASBA amplification and Kenten does not teach or suggest the desirability of using NASBA amplification with ECL detection. The differences between NASBA and PCR reactions are substantial and the skilled artisan would not reasonably expect that the successful incorporation of ECL technology with PCR would be a good predictor of success with NASBA. Thus, one of ordinary skill in the art would not have the motivation to combine teachings of Malek and Kenten for the reasons set forth above.

With respect to (C), there is no motivation or suggestion in any of the references to make the combination suggested in the Final Office Action.

With respect to (D), there is no reasonable expectation of success in making a combination suggested by the Examiner due to the differences between NASBA and PCR reactions as discussed above.

Favorable reconsideration and withdrawal of the Section 103(a) rejection are earnestly solicited.

CONCLUSION

In view of the foregoing, Appellants respectfully submit that the instant claims are non-obvious over Malek in view of Kenten. Thus, the claims are in condition for allowance and such action is earnestly solicited.

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Respectfully submitted,

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APPENDIX A

32. A process for the detection of a specific nucleic acid sequence comprising:

(a) forming a composition comprising

(i) a sample,

(ii) a first oligonucleotide primer which comprises a promoter sequence,

(iii) a second oligonucleotide primer,

(iv) a DNA-directed RNA polymerase,

(v) an RNA-directed DNA polymerase,

(vi) a DNA-directed DNA polymerase, and

(vii) a ribonuclease that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA;

(b) incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence to form an amplified nucleic acid sequence mixture comprising an amplified nucleic acid sequence;

(c) forming a second mixture by adding to a sample of said amplified nucleic acid sequence mixture the following reagents

(i) at least one detection probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said detection probe sequence being labeled with an electrochemiluminescent species,

(ii) at least one capture probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said capture probe sequence being labeled with a binding species, and

(iii) a solid phase coated with a binding partner of said binding species;

(d) incubating said second mixture for a time sufficient to allow hybridization of said probes to said amplified nucleic- acid sequence and to allow binding of said binding species to said binding partner so as to form a solid phase-bound hybridization complex; and

(e) detecting said solid phase-bound complex by using said electrochemiluminescent species.

33. The process of claim 32, wherein said solid phase is a magnetic bead.

34. The process of claim 32, wherein the binding species/binding partner pair are selected from the group consisting of biotin/avidin, biotin/streptavidin, and digoxigenin/anti-digoxigenenin.

35. The process of claim 32, wherein the binding species is biotin and the solid phase is a streptavidin-coated magnetic bead.

36. The process of claim 32, wherein said amplified nucleic acid sequence is the anti-sense copy of the specific nucleic acid sequence and wherein said amplification of said specific nucleic acid sequence is carried out under conditions which permit

(i) said second oligonucleotide primer to hybridize to an RNA template which comprises the specific nucleic acid sequence or an anti-sense copy of the specific nucleic acid sequence,

(ii) said RNA-directed DNA polymerase to utilize said RNA template to synthesize a DNA template by extension of said second oligonucleotide primer and thereby form an RNA-DNA hybrid intermediate,

(iii) said ribonuclease to hydrolyze RNA contained in said RNA-DNA hybrid intermediate,

(iv) said first oligonucleotide primer to hybridize to said DNA template,

(v) said DNA-directed DNA polymerase to utilize said DNA template to synthesize a double-stranded DNA product by extension of said first olignucleotide primer, said double stranded DNA product comprising said promoter, and

(vi) said DNA-directed RNA polymerase to recognize said promoter and transcribe said double- stranded DNA product so as to form more RNA first template.

37. The process of claim 32, wherein said electrochemihuninescent species comprises ruthenium-tris-bipyridine.

38. A process for the detection of a specific nucleic acid sequence, comprising:

(a) forming a composition comprising:

(i) the sample,

(ii) a first oligonucleotide primer which comprises a promoter sequence,

(iii) a second oligonucleotide primer,

(iv) a DNA-directed RNA polymerase,

(v) an RNA-directed DNA polymerase,

(vi) a DNA-directed DNA polymerase,

(vii) a ribonuclease that hydrolyzes RNA of an RNA-DNA hybrid without hydrolyzing single or double-stranded RNA or DNA, and

(viii) one or more nucleotides;

(b) incubating the reaction mixture for a sufficient time to amplify said specific nucleic acid sequence to form an amplified nucleic acid sequence mixture comprising an amplified nucleic acid sequence;

(c) forming a second mixture by adding to a sample of said amplified nucleic acid sequence mixture the following reagents

(i) at least one detection probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said detection probe sequence being labeled with an electrochemiluminescent species,

(ii) at least one capture probe sequence which specifically hybridizes to said amplified nucleic acid sequence, said capture probe sequence being labeled with a binding species, and

(iii) a solid phase coated with a binding partner of said binding species;

(d) incubating said second mixture for a time sufficient to allow hybridization between said probes and to allow binding of said binding species to said binding partner so as to form a solid phase-bound hybridization complex; and

(e) detecting said solid phase-bound complex by using said electrochemiluminescent species;

wherein, optionally, said first primer, second primer or at least one of said nucleotides is labeled with the electrochemiluminescent species and said detection probe is omitted, and, optionally, said first primer, second primer or at least a portion of said nucleotides is labeled with the binding species and said capture-probe is omitted.

39. The process of claim 38, wherein the solid phase is a magnetic bead.

40. The process of claim 38, wherein the binding species/binding partner pair are selected from the group consisting of biotin/avidin, biotin/streptavidin, and digoxigenin/anti-digoxigenenin.

41. The process of claim 38, wherein the binding species is biotin and the solid phase is a streptavidin-coated magnetic bead.

42. The process of claim 38, wherein said amplified nucleic acid sequence is the anti-sense copy of the specific nucleic acid sequence and wherein said amplification of said specific nucleic acid sequence is carried out under conditions which permit

(i) said second oligonucleotide primer to hybridize to an RNA template which comprises the specific nucleic acid sequence or an anti-sense copy of the specific nucleic acid sequence,

(ii) said RNA-directed DNA polymerase to utilize said RNA template to synthesize a DNA template by extension of said second oligonucleotide primer and thereby form an RNA-DNA hybrid intermediate,

(iii) said ribonuclease to hydrolyze RNA contained in said RNA-DNA hybrid intermediate,

(iv) said first oligonucleotide primer to hybridize to said DNA template,

(v) said DNA-directed DNA polymerase to utilize said DNA template to synthesize a double-stranded DNA product by extension of said first oligonucleotide primer, said double stranded DNA product comprising said promoter, and

(vi) said DNA-directed RNA polymerase to recognize said promoter and transcribe said double stranded DNA product so as to form more RNA first template.

43. The process of claim 38, wherein said electrochemiluminescent species comprises ruthenium-tris-bipyridine.